IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	LOSKUTOFF ET AL.	Examiner:	V. AFREMOVA
Serial No.:	10/044,796	Group Art Unit:	1651
Filed:	OCTOBER 11, 2002	Docket No.:	13511.1USU1
Title:	SEMEN EXTENDER COMPOSITION AND METHODS FOR MANUFACTURING AND USING		

Declaration under 37 C.F.R. §1.131

I, Richard B. Lomneth, Ph.D. declare as follows:

1. I am one of the originally named inventors of the above-identified patent application.

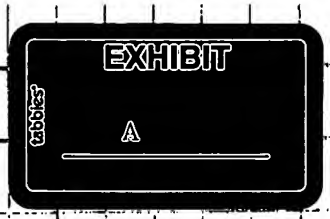
2. Attached as Exhibit A are pages 3-6 from a laboratory notebook. These pages have been redacted to cover the dates in the upper right hand corners. The dates are all prior to May 14, 1999. The handwriting on the pages of laboratory notebook in Exhibit A is mine, and the reported compositions were prepared by me prior to May 14, 1999.

3. Sample 2 reported on laboratory notebook page 6 provided as Exhibit A identifies a sample I prepared containing 1 wt.% lecithin, 0.5 ml Biladyl® concentrate, 0.1 wt.% Equex, and water to 5 ml. To the 5 ml composition, 0.7 ml glycerol was added. Biladyl® is available from Minitüb GmbH, Germany. Biladyl® contains carbohydrate and buffer. Attached as Exhibit B is a product sheet for Biladyl®. In addition, Biladyl® is identified by the above-identified patent application at, for example, page 14, lines 15-18. Equex contains sodium lauryl sulfate as a surfactant and is identified by the above-identified patent application at page 16, line 11 through page 17, line 8 wherein "EQ" refers to Equex.

4. Sample 2 reported on laboratory notebook page 6 in Exhibit A describes a composition containing the components of independent claims 1 and 21 of the above-identified patent application. The phospholipid obtained from a non-animal source is satisfied by the lecithin, the surfactant to reduce ice crystal formation during freezing of the composition is satisfied by the sodium lauryl sulfate, the carbohydrate and the biological buffer are satisfied by the Biladyl® component, and the freeze agent is satisfied by glycerol. It is my belief that the composition of sample 2 exhibits a pH of about 6.9 to about 7.5 and an osmolality of about 250 mOsM to about 350 mOsM.

5. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: March 29, 2005 Richard B. Lomneth, Ph.D.



START MAKING SAMPLES FOR TESTING

12-22 BILA 4.5% LECITHIN "BEEVO BUFFER BUFFER"
12-23 " 3.5% " " " "
12-23 " 1% " " " "

- LECITHIN DISPERSED IN H_2O, THEN DILUTED INTO BILAYOL A

BILAYOL A - "10x" SOLUTION (140mL) DILUTE 400mL

SO LECITHIN PROPERLY DISPERSED IN H_2O ~~IN H~~ FOR DILUTION W/ BILA CONCENTRATE
BILA = ADDITIVES MIXED 1:1 WITH SPERM + COOLED
BILB = BILA, DILUTED WITH GLYCEROL (8% LIKELY). MIX 1:1 WITH SPERM/BILA MIXTURE

EQUEX PASTE "9-98"
CONCENTRATION = ? CALL MINI TUBE

DILUTE SEMEN IN HEPES TL
↳

NEED A BILAYOL A STOCK

GENERAL PLAN:
1. MAKE A HEPES TL ANALOG FOR DILUTION OF SEMEN SO ALWAYS SAME LECITHIN : SEMEN DILUTION
2. MAKE LECITHIN; BILA, H_2O AT HIGHER THAN FINAL CONCENTRATION; DILUTE IN BILA 300mOsm AS NEEDED
3. MAKE LECITHIN-GLYCEROL STOCK SO THAT LECITHIN IS AT CORRECT FINAL CONCENTRATION (14% GLYCEROL RECOMMENDED BY DEVIN VALENCIA)
4. MAKE SAMPLES AT "PSEUDO CONCENTRATIONS" FOR THIS WEEK.

1. Make 0.1% Equex Paste in Lecithin Solution
   a. Make 10mL 1% Lecithin, 0.1% Equex
   ~~b. 2mL 5% Lecithin~~
   b. 10mL 1% Lecithin
      0.1% Equex Paste: $C_1V_1 = C_2V_2$
      $(10mL)(0.1\%) = (100\%)(V_2)$
      10λ Equex
   c. Label "1% Lecithin, 0.1% Equex"

2. Cholesterol / Methyl-β-Cyclodextrins
   a. Use ~5mg/mL Ch – Methyl β Cyclodextrin with Spoon
   b. For 5mL Sample, Need 25mg Cyclodextrin (= Methyl-β-Cyclodextrin = MβCD
   c. Stocks – 30mg Cholesterol / 1g Cyclodextrin
   d. ∴ Make 10mL in H_2O
      (5% Cyclodextrin)(10mL) = .5g Cyclodextrin
      Add ~15mg Cholesterol
      Add H_2O to 15mL Tube, Add 0.50 g Methylated β Cyclodextrin Cerestar Lot I 8044
      (– Goes into Solution Easily)
   e. Cholesterol: Sigma C-3045 Lot 84H84585
      43.8mg Cholesterol
      Add 973μL Isopropanol (+F 45mg/mL is Dissolved)
      Dissolved at Room Temp w/ Vortexing
   f. If 30mg Cholesterol = 667μL
      ∴ total 10.67mL Has 500mg Cyclodextrin + 30mg Cholesterol
      ~~If Want 5mg/mL~~
      Want 25mg Cyclodextrin / Aliquot 46.86mg/mL
      $\left(\frac{46.86mg}{mL}\right) = \frac{25.0mg}{X}$ $X = 0.534mL$ / Tube = 20 Tubes
   g. Add Cholesterol to MβCD
      – Slow Access – Find Way To Improve Temp Control Mixing
      – Should Have Added 15mg Cholesterol!!!
      – First 15mg Went In Readily; All But 0.080mL Added } It Almost All Went Into Solution
      – Spin Sample To Remove Undissolved Cholesterol, Store Aliquot ~~0.533mL/Tube~~ 400μL
      Not Sure Everything Dissolved + Speed Vac / Final Vol = 2.0mL 355μL/Tube

TEST SOLUBILITY OF CH-MBCA IN H_2O, THEN DILUENT
- APPEARS TO COMPLETELY DISSOLVE IN 1.0mL H_2O.
- ADD TO LECITHIN / BILAYR
  - APPEARS TO COMPLETELY DISSOLVE IN 1.0mL 1% LECITHIN IN BILAYR

- MAKE 5% PGE (Polyglycol Ester) (SOLID, RBL) IN H_2O
  5g/100mL DO 2.5g/50mL OR 2.0g/40mL
  (2.02g) ADD 40.0mL H_2O

HEAT IN A MICROWAVE — OVERFLOWED. MICROSCOPICALLY LOOKED WELL DISPERSED. STILL HAS HIGH TEMP (NOT MEASURED TEMP)
- BETTER DISPERSED.

- MAKE 5% PGE IN BILAYR A (1/29 w/ ANTIBIOTICS)
  2.0g PGE (SOLID) RL / 40.0mL BILAYR A

HEAT (IN MICROWAVE), SHOW ~2MIN, SETTING 8
↳ HOT (NOT MEASURED) TOO HOT TO HOLD COMFORTABLY

TENTATIVE PROTOCOL FOR WORKING WITH SAMPLES.

1. MAKE SURE SEMEN HAS ≥25x10⁶ MOTILE SPERM; >50% MOTILITY
2. MIX SEMEN WITH EXTENDER, 1:1 RATIO
3. ~~COOL~~
4. ~~MIX W/ 2ND SET OF EXTENDER CONTAINING 14% GLYCEROL~~
3. FREEZE

$(14\%)\frac{5t}{(x)} = \frac{x}{5+x} \times 100 = 14$

$\frac{x}{5+x} = .14$

$\frac{x}{5+x} = .70$

$.86x = .70$

$x = \frac{.70}{.86}$

ADD 0.80mL GLYCEROL

PGE APPEARS TO HAVE PRECIPITATED OUT OF SOLUTION. MAY HAVE TO TRY A LOWER MW (MORE SOLUBLE) FORM

SAMPLES

✓ 1. ~~5~~ 5mL 1% LECITHIN/BILADYL + 0.7mL GLYCEROL (5mL 1% LEC/BIL FROM 12.23)

✓ 2. 5mL 1% LECITHIN/BILADYL + 0.1% EQUEX + 0.7mL GLYCEROL (5mL 1% LEC/EQUEX/BIL FROM 3.15.99)

✓ 3. 5mL 1% LEC/BIL + 0.7mL GLYCEROL - ADD 1mL TO Ch-MβCD TUBE + DISPERSE + MIX WITH REMAINDER OF 1% LEC BILADYL (5mL 1% LEC/BIL FROM 1.23)

✓ 4. 5mL 1% LEC/BIL + 0.1% EQUEX + 0.7mL GLYCEROL - ADD ~~½~~ 1mL TO Ch-MβCD TUBE, DISPERSE + MIX W/ REMAINDER (5mL 1% LEC/EQUEX/BIL FROM 3.15.99)

✓ 5. SAME AS "3" EXCEPT USE 2 TUBES Ch-MβCD (5mL 1% LEC/BIL FROM 12.23)

✓ 6. 5mL 4% PGE, 1% LEC/BIL + 0.7mL GLYCEROL (4mL 5% PGE/BIL 3.15.99 + 1mL 5% LEC 12.22)

✓ 7. 5mL 2% PGE, 1% LEC/BIL + 0.7mL GLYCEROL (2mL 5% PGE/BIL 3.15.99 + 1mL 5% LEC 12.22 + 2mL BIL 1.29)

✓ 8. 5mL 4% PGE, 1% LEC/BIL + 0.1% EQUEX + 0.7mL GLYCEROL (SAME AS 6 PLUS 5µL EQUEX

✓ 9. 5mL 2% PGE, 1% LEC/BIL + 0.1% EQUEX + 0.7mL GLYCEROL (SAME AS 7 PLUS 5µL EQUEX

✓ 10. 5mL 4% PGE, 1% LEC/BIL + ADD TO Ch-MβCD TUBE AS ABOVE (SAME AS 6 PLUS Ch-MβCD.

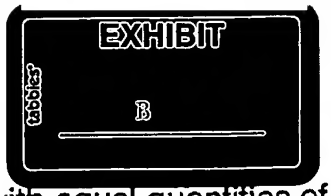
✓ 11. 5mL 2% PGE, 1% LEC/BIL + ADD TO Ch-MβCD TUBE AS ABOVE (SAME AS 7 PLUS Ch-M

✓ 12. 5mL BILADYL NO LECITHIN + 0.7mL GLYCEROL (5mL BIL A w/ ANTIBIOTICS 1.29)

✓ 13. 5mL 4% PGE, 1% LEC/BIL, 0.1% EQUEX, ADD TO Ch-MβCD AS ABOVE (SAME AS 8

✓ 14. 5mL 2% PGE, 1% LEC/BIL, 0.1% EQUEX, ADD TO Ch-MβCD AS ABOVE (SAME AS 9 PLUS Ch-MβCD)

BILADYL®



Preparation of COCKTAIL AB:

Add 12 ml of double distilled, sterile water, using a sterile syringe.

Final composition of reconstituted COCKTAIL AB expressed as active units of antibiotics per 0.02 ml:
100 µg Tylosin,
500 µg Gentamicin,
300 µg Lincomycin,
600 µg Spectinomycin

Usage for „Neat Semen Treatment":
Add and carefully mix 0,02 ml to each ml of neat semen, using a sterile syringe.

Usage for BILADYL SOLUTION A:
Add and carefully mix 10 ml to SOLUTION A, using a sterile syringe.

Preparation of SOLUTION A:

1) Reconstitute 49 g of SOLUTION A with double distilled sterile water to a combined volume of 390 ml.
2) Add 100 ml clean yolk from fresh chicken eggs.
3) Add 10 ml of reconstituted antibiotics COCKTAIL AB, using a sterile syringe.
4) Mix gently and warm mixture to + 30° C (+ 86° F)
5) Filter medium before adding it to semen.

Preparation of SOLUTION B:

1) Reconstitute 250 g of SOLUTION B with double distilled sterile water to a combined volume of 400 ml.
2) Add 100 ml clean yolk from fresh chicken eggs.
3) Mix gently and warm mixture to + 30° C (+ 86° F)
4) Filter medium before adding it to semen.

Usage:
Dilute semen with equal quantities of SOLUTION A and B according to the CSS Processing Regulations.

Final composition of SOLUTION A and B per 100 ml, as approved by CSS:
Yolk 20 %, Glycerol 7 %, Tris 2,42 %, Citric Acid 1,38 g%, Fructose 1,00 g%,
Active Units of Antibiotics:
Tylosin 5,25 mg, Gentamicin 26,25 mg, Lincomycin 15,75 mg, Spectinomycin 31,5 m
and double distilled sterile water

Storage:
At a controlled temperature of + 5° C (+ 41° F) in a dark environment.
Shelf life: 12 months.

Warning:
Keep out of reach of children
Not for human or animal consumption and/or treatment
Not for injection
Not for use on live animals
Do not expose to heat or sun

Made in Germany

BILADYL IS APPROVED BY CERTIFIED SEMEN SERVICES INC.

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minitüb

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